Attachment 1

DEVELOPMENTS IN FOOD SCIENCE 27

CHEMISTRY AND ANALYSIS OF HOP AND BEER BITTER ACIDS

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CHAPTER 1
HOPS AS A RAW MATERIAL IN THE BREWERY

1.1. OCCURRENCE OF HOPS.

Hops are grown for commercial purposes in most of the moderate climate zones of the world. Hop growing areas are situated between latitudes 43°-54° (Europe), 38°-51° (North America), 38°-51° (Japan) in the Northern hemisphere and between 37°-43° (Australia), 41°-42° (New Zealand), 35°-40° (Argentina) in the Southern hemisphere. Wild growing hops are also found in these parts of the world.

The largest hop growing areas are situated in the South-East and Mid-West of England, the Saaz and Auscha districts of Czechoslovakia, the Hallertau region of Germany, the Slovenian parts of Yugoslavia and in the states of Washington, Oregon and California in the United States of America.

Hop needs a fertile soil and specific climatic conditions, especially with respect to the length of the days and the summer temperature. The amount of rain and groundwater is also rather critical for successful cultivation of hop. The hop is therefore one of the most difficult plants to raise.

1.2. ORIGIN AND HISTORY.

Hop or *Humulus lupulus* finds its origins as a wild plant in Europe and Western Asia. Plinius in his "Naturalis Historia" mentions the hop as early as the first century A.D. as a garden plant, grown for its flowers and for its shoots, and which were (and are) eaten as a kind of asparagus. Hops apparently grew between the willow trees "like a wolf between sheep". This explains the name *'Lupus salictarius'* the Romans used, from which the later name *Humulus lupulus* is derived. Hop was used in brewing by the Sumerians and Egyptians as far back as 3000 years ago. The Babylonians and Philistines knew beer, but whether they used hops is not certain.

Hop then disappeared from the scene and only came back much later. Pepin the Short mentions hops in a Donation Act of the year 768. In the 8th and 9th century, cloisters and monasteries (for example the Abbey of Freising in Bavaria) possessed large hop gardens. Hops were then primarily used for medicinal purposes. Documents of the year 822 indicate that monks of Picardy reintroduced the use of hops in brewing when they founded the Corbay cloister on the Weser in Northern Germany. From the 12th and 13th century hops were used extensively in the breweries of Germany. The

name of the plant was then 'Hopfo'. There were strong feelings against hops, since traditionally brewers used grits, rosemary and other aromatic herbs to flavour beer.

The cultivation of hops was introduced in Flanders in the 14th century, and was encouraged by the Order of the Hop, initiated by John the Fearless, Duke of Burgundy. For a long time it has been thought that hops growing was imported into England in the 15th century, via the trade contacts with the Flemish weavers. However, plant fragments, found in the remnants of a boat sunk in the year 949 near Graveney, Kent, show that hops were already known in England at that time (1). Several English books refer to the hop growing activity in Flanders and to the transfer of hop growing know-how to the English growers. The importance of hop as a brewing material steadily increased in Europe (2,3).

The introduction of hops in America by the Massachusetts Company dates from 1629 but commercial growing started only in the 19th century. Growing hops in Japan and in the USSR commenced even later.

Through the ages the use of hops in brewing gradually gained acceptance. Today, all beer is treated with hops or with hops-derived products. It is estimated that beer consumption per head in the Middle Ages in Western Europe was about 5 to 10 times higher than it is today. This probably had to do with the absence of cheap alternatives and the lack of drinking water of good quality. In this context it must be noted that milk and wine were a luxury and that coffee and tea were not yet known.

The number of fermented beverages known today all over the world is large indeed. It is the hop which imparts to beer the characteristic flavour, setting beer apart from all other drinks, and thus making hop the most essential and indispensable raw material in the brewery.

1.3. HOP AS A BOTANICAL SPECIES.

1.3.1. DESCRIPTION OF THE HOP PLANT.

The hop belongs to the plant family of the Cannabinaceae (4), consisting of the two genera : Humulus and Cannabis. Humulus has two species, *Humulus lupulus L.* and *Humulus japonicus Sieb. & Zucc.* All cultivated hops are varieties of *Humulus lupulus. Humulus japonicus* has no brewing value and is grown only as an ornamental plant.

The hop is a dioecious plant with unisexual male and female flowers growing on separate plants. For commercial purposes seeds in hops are considered to be a negative quality criterion and growing male plants is therefore rigorously controlled. At one time there was a clear difference on this point between continental and English hops, the English hops being readily seeded.

Hop plants have a fair resistance to frost as the root system is very large and burrows deep into the ground. This is necessary during the period of very rapid growth. New shoots of the roots are sometimes harvested and eaten as a culinary delicacy resembling asparagus.

The hop bine or hop vine climbs on anything available, turning in a clockwise direction. In the commercial growing of hops, a suitable support is provided in the form of poles or a network of wires, or both. The curvature of the helix of the growing vine is constant and therefore the steepness of the growth is the higher the diameter of the support becomes smaller. The vine has a diameter of about 1 cm and is hexagonal in section. Strong hooked hairs grow on this stem and fix it to the support. The colour of the stem is a varietal characteristic. The length of the stem is usually around 6 to 10 m.

Branching occurs from buds in the axils of the leaves on the main vine. The leaves of the hop grow in opposing pairs from nodes on the main vine. Most of the leaves have three or five lobes. Sometimes three- and five-lobed leaves occur on the same plant. The leaves are hairy on both sides and they can reach up to 20 cm in length. The colour is green to yellowish-brown.

1.3.2. DEVELOPMENT OF THE HOP CONE.

Only female hop flowers are important for commercial purposes. Flower growth starts in June-July by budding in the axils of the leaves of the lateral branches and at the top of the main vine. The buds form short stalks which are called 'burrs'. The central axis carries alternate pairs of very small bracts. These are vestigial leaf structures and occasionally a real leaf is formed between the bracts. In some hop varieties this is quite common (e.g. Northern Brewer). In the hop flower so-called lupulin glands are formed on the flower leaves and at the base of their attachment to the central axis of the flower or cone. In these yellow, golden-brown lupulin glands the 'resins', which comprise the hop bitter acids, are secreted. Lupulin glands are only weakly attached to the hop flower and, therefore, hops have to be handled carefully in order not to lose precious lupulin.

Seeded hops are heavier than unseeded hops because of the weight of the seeds, which can reach 1/3 of the cone weight. Seeds contain 33% fats and oils (5). They have no brewing value and are often even considered as a negative hop quality factor (6-8). Seed formation is nowadays avoided as much as possible by eliminating all fertilizing male hop plants. Growing of male hop plants is forbidden by law and wild growing plants have to be destroyed.

1.3.3. GROWING AND PICKING OF HOPS.

In spring, when hops start to grow, the shoots are restricted to a number ranging from 4 to 12. By June the plant is 3-4 m long and in July-August it reaches 6-10 m. Hop is therefore one of the fastest growing plants. Full flowering is reached in about 2-3 weeks while a further 3-4 weeks are required before the optimum resin concentration in the lupulin glands is reached. The time of picking is most important for determining the quality of a hop variety.

In the Northern hemisphere the earliest varieties are picked at the end of August or the beginning of September. About half the weight of the above-ground visible plant consists of cones. Some hop varieties are picked as late as October. In November the vine withers away while the underground root system continues to take up nutrients and water. In the Kashmir valley of India two crops per year can be raised, but the total yield is not greater than elsewhere (9).

Ideally, the best period for picking hops should be determined by a daily alpha acids analysis of the flower cones on the vines. In practice, the picking time is determined by visual examination of the cones, by weather conditions, by commercial considerations, and nowadays by the availability of the machines used to separate the cones from the vines. The vines are still cut by hand and then brought to the cone picking machine; the lay-out of modern hop fields takes this into account

In the not so distant past, picking of the hop was carried out by hand by temporary workers who stayed in the fields for weeks. At the end of the picking time festivities were usual and these have been often depicted in paintings and other works of art.

Very early picked hops (perhaps too early) have a green colour and a fresh smell. However, the hop bitter acids and essential oil content (specifically the alpha acids content) is then low or even very low. This is the case for the Saaz variety and to a lesser extent also for other varieties of Central Europe. Although these hops have only a low alpha acids content, they are considered to be of high quality.

Hops which are picked later, at the height of their alpha acids content, may already have reached a stage where end-of-season oxidative deterioration has started. The essential oil content of such hops will also be high and the composition of the essential oil may reflect a relatively high state of oxidation. Such hops have a riper aspect and smell and are often designated as bittering hops. Although their alpha acids content is high, their quality estimation may be low. Often these hops are used to produce extracts.

1.3.4. DRYING OF HOPS.

As already noted, hops are difficult to grow as they need constant attention, fertile soil, plenty of water and a sunny period of at least 2-3 weeks just before picking, to ensure a crop of good quality and high yield. Freshly picked hops contain about 80% water and cannot, therefore, be stored as such. Drying hops, without loss of quality and of lupulin glands, needs special installations called kilns. These kilns form part of purpose-built structures which are sometimes called oast houses.

The fresh hops to be dried are placed in recipients with a wire netting bottom and warm air is blown through the stacked containers until the water content of the hops has been reduced to about 5-6%. Whilst cooling off, the hops are allowed to pick up some air moisture again (up to 10% in weight) without the hops being taken out of the containers in order to avoid lupulin loss which can be severe in the very dry state.

The drying temperature is mostly between 55 and 70°C and the drying time is around 10 hours. Drying has a profound effect on the aspect and quality of the hops. To avoid yellow-brown colouring of the hops, sulfur is sometimes burned in the drying gasstream. This procedure causes bleaching of the hop colour and also influences the total sulfur content (10); whether this is a positive factor or not has been debated. The drying process is thermally inefficient and the drying is inhomogeneous as the lower layers of hops are heated at higher temperatures and dry more quickly than the upper layers. Improvement could be expected from a continuous process, but the hops drying installation is only used when it is required during a few weeks per year at the most, so that capital investment for such a continuous drying installation is a delicate matter.

Hop storage in the brewery is also a much studied topic. Hops are generally stored in cooled rooms. Whether this is really necessary has been strongly contested. A great number of papers have been dedicated to the problems of hop storage. A fairly recent contribution by Forster (11) describes the keeping characteristics of pelletized hops. This reference can help to trace earlier work in the field of hop storage.

1.3.5. HOP VARIETIES.

There exist many hop varieties and the search for newer types with improved characteristics still goes on. Some well known varieties are Northern Brewer, Hallertau, Saaz, Brewers Gold, Bullion and Jakima. Newer varieties are especially studied at Wye College, Ashford, Kent in England (10).

Varieties can be named according to the name of the region where they are cultivated (Saaz, Wye) or from the name of the person who first introduced them (Fuggle, Golding). Confusion can arise because, for example, a Northern Brewer

variety raised in the Hallertau region of Germany may be called a Hallertau hop.

Developing new hop varieties is fairly complicated. Plant characteristics are governed by the chromosomes present in the nucleus of each plant cell. The number of chromosomes in a cell is 20, except in the male pollen grain cell and in the egg cell of the female flower where the number is only 10. On fertilization the chromosomes of the sexual cells fuse and again total 20 (diploid) in the embryo formed. This makes it difficult to maintain an acquired character which may be lost on each such cycle, since a number of chromosomes are not present in the sexual cells. A way round this difficulty is to block splitting of the chromosomes in two groups of 10. This can be achieved by chemical treatment with, for example colchicine. The sexual cells of plants treated in this way contain 20 chromosomes and the offspring has 40 chromosomes (tetraploid). When a normal female egg cell of such a tetraploid, with 20 chromosomes, is fertilized by male pollen with 10 chromosomes, the result is a triploid with a larger probability that this will retain the female plant characteristics since it contains all the female plant chromosomes. By crossing tetraploid female plants with hardy, resistant diploid male plants a triploid variety is obtained with high disease resistance, while still retaining all the possibly desirable characteristics of the female plant. Triploid hop plants are sterile, but they can be bred by multiplying shoots, with full retention of the characteristics.

To assure consistency and uniformity of brewing value, the multiplication of hop plants for large-scale growing is always carried out by shoot cultivation (2). The base of the vine is buried in the fall and new roots and shoots form, which can be planted in the spring. Shoot formation is also possible from other plant parts such as the lateral branches on the vines.

Rigby has argued that a high cohumulone percentage is a negative quality factor (12). Although the evidence presented is rather circumstantial, his arguments are quite convincing. On the other hand, a low non-oxidized essential oil content would be favourable. This would explain the value attributed to some European hop varieties which have both these characteristics (low oil an low cohumulone content). In this context it is of interest to mention that nowadays the ratio of cohumulone in the alpha acids and the essential oil content can be controlled at will (13). The authors of this paper state :

"By using selected lines and varieties for hop breeding, it is now possible to combine high cone yield and high alpha acids content, modify the alpha and beta acids content and proportions, combine high alpha acids content with superior storage stability, create an array of varieties with cohumulone content from <20% to >65%, independently of alpha acids content, select lines differing in essential oil content by a factor of up to 4, and significantly modify the composition of the essential oil".

It seems therefore that any required hop quality can now be produced almost at will (13,17).

Perhaps a high cohumulone content in itself is not a bad quality factor, but is related to other negative factors present in hops grown for a high alpha acids content. Such hops are picked when really ripe and, therefore, the oxidation state is also fairly advanced. This is considered to be less important, since the hops are grown for a high alpha acids content. The quality of the so-called aroma hops, such as Saaz, would be related to very early picking. This results in a low alpha acids and low, non-oxidized essential oil content. It should be worthwhile to experiment with 'unripe high cohumulone content' hop.

Another point of long standing interest is the desire to recognize and characterize different hop varieties by chemical analysis. Pattern recognition by efficient Gas or Liquid Chromatography (GC or LC) of bitter acids, of the polyphenols, or of the essential oils, has been studied extensively. The most recent contributions to this aspect of hop research recognize the difficulty of this proposition and warn against over-optimism (14-16). It may also be expected that commercial interests will lead to mixing of varieties, in order to attain certain desirable analysis patterns. This would render varietal recognition by chemical analysis practically impossible.

1.4 HOP AS A COMMERCIAL PLANT.

1.4.1 THE HOP MARKET.

There are about 25 countries where hops are cultivated. The largest hop grower is Germany with 30% of the total world production, followed by the USA with 20%, the USSR and Czechoslovakia with each 10% and England with 6%. A brisk and important trade is maintained between the large hop growing countries (Germany, USA, Czechoslovakia, Yugoslavia, Australia, China) and the beer producing countries which do not cultivate hops (The Netherlands, Denmark, Brazil, Mexico, the Comecon countries, some African countries). Large amounts of hops are also exchanged between hop growing countries because of the quality differences of the different varieties.

World production of hops; measured in alpha acids weight, was largest in 1973 with 7500 tonnes, exceeding world demand by about 1000 tonnes. This excess was the result of increasing surface area of cultivation until 1974, of higher crop yields per hectare and of the higher alpha acids content of the new varieties. The demand for

hops increased only very slowly with increasing beer production because of more rational use and also because of the tendency towards less bitter beers in most parts of the world. The increasing interest in alcohol-free beer, which requires less hops, also contributed to this situation.

World reserves soon were larger than about half the yearly requirements. This situation had disastrous consequences for hop growers all over the world. In Belgium for example, the number of growers was halved in less than 5 years (18). This overreaction predictably led to a revival of the health of the hop-market. Now, in 1991, demand for hops is brisk again and prices are relatively high. In this context it is of importance to acknowledge the increasing beer consumption in the developing countries and especially in South America. Hops have, however, always had a bad reputation of wildly fluctuating prices and there is little reason to believe that this will not continue in the future.

1.4.2 COMMERCIAL USE AND COMPOSITION OF HOPS.

The hop only became generally accepted for bittering beer in the 19th century. Originally, hops were used not so much to flavour beer, but to improve its preservation time; in other words, hops were used for their 'preservative value'. As production became more and more centralized, beer had to be transported over longer distances and required longer preservation periods. Pasteurization had not yet been introduced as an industrial practice and therefore the use of hops was essential for its preservative effect. Although this particular aspect in no longer relevant, the hop is now established as an important raw material in the brewery.

About 1/5 of the dry hop weight consists of lupulin particles. These contain all the important components of hops, the bitter acids and the essential oil. The hop bitter acids comprise the alpha and the beta acids. These are only crystalline in very pure form. As mixtures they present themselves as oils or resins which are soft and soluble in hydrocarbon solvents (soft resins). Through air oxidation the hop bitter acids are transformed into ill-defined products which are not longer soft and soluble in hydrocarbons (hard resins). Hops contain yet other organic and inorganic compounds and mixtures. The composition of hops is presented in Table 1.

Evaluation of hops was and is still carried out on smell and appearance of selected small samples. To improve objective assessment, determination of soft and hard resins, by dissolving experiments, was at one time a generally applied method. Today the most important analytical determination for hop evaluation is the quantitative analysis for the alpha acids. This is discussed fully in chapters 15 to 17.

Table 1. Composition of hops.

Nature	Weight %
Alpha acids	2-12
Amino acids	0.1
Beta acids	1-10
Cellulose	40-50
Chlorophyll	-
Essential oil	0.5-5
Monosaccharides	2
Oils and fatty acids	Traces to 25%
Pectins	2
Polyphenols (tannins)	2-5
Proteins	1
Salts (ash)	10
Water	8-12
Waxes and steroids	-

1.4.3. HOPS IN THE BREWERY.

Since hops are subject to oxidative resinification of the bitter acids, they are usually protected by cold storage. Practically all breweries therefore possess a cold room where the hops are stored. A large number of studies has been devoted to the problem of hop storage and deterioration with time. After an induction period of some weeks or months, during which changes are small, the hop bitter acids content diminishes exponentially (19,20). Important factors are the temperature and the composition of the atmosphere in contact with the hops (21,22). Hop deterioration can be slowed down by enclosure in airtight bags at low temperatures, preferably 0°C or even lower. Claims have been advanced that the loss of alpha acids does not reflect the loss in bittering potential of deteriorated hops (23). This must be caused by a contribution to beer bitter taste by the oxidation products of the alpha acids and probably also of the beta acids. That old, oxidized hops, containing little or no detectable alpha acids can bitter beer, has been ascertained repeatedly. It has therefore been stated that hops must not necessarily be kept refrigerated. On the other

hand, the negative influence of oxidation on the smell of the hops is detectable in beers brewed with such hops. Obviously there is much controversy around this topic among brewers. We believe that the use of old or oxidized hops may be acceptable in heavy, dark beers with a strong taste background (top fermentation), but not for lighter, lager-type beers (bottom fermentation).

Hops or hops-derived products are usually boiled for 0.5 to 2.5 hours with wort. Wort is an aqueous solution of fermentable sugars (glucose, maltose, maltotriose) obtained by enzymatic hydrolysis of starch derived from malt extraction or from other sources (wheat, maize, rice). The hop boiling takes place in copper or stainless steel tanks and aims to precipitate wort proteins, to sterilize the wort, to remove volatile off-odours by steam distillation and most importantly, to transform the alpha acids into beer-soluble and bitter iso-alpha acids.

Depending on the wort density, the hop variety and its alpha acids content, 100 to 800 g hops or a hops-derived equivalent is added per hectolitre. This addition is not always made at the beginning of the wort boiling process. Often, alpha-rich or bitter hop is added at the start, while a smaller amount of so-called noble or aroma hop is added only 15 to 30 minutes before the end of wort boiling. The aim is to obtain the necessary bitter taste level by fractional addition of alpha-rich and cheaper hops in the first stages of the wort boil and to achieve a desirable aroma contribution of the more expensive aroma hops by reducing the time during which essential oils or other aroma components are removed from the boiling wort by steam distillation (24).

After wort cooling and filtering, yeast is added to ferment the sugars to ethanol and carbon dioxide. Top fermentation occurs at 15-20°C while the yeast rises to the surface, which induces oxidation reactions colouring the beer (*Saccharomyces cerevisiae*). Bottom fermentation occurs at 5-9°C or 7-13°C while the yeast flocculates to the bottom of the fermentation tank (*Saccharomyces carlsbergensis*). When the fermentation is finished, the yeast is removed and the beer is stored for a certain time in lagering tanks, before being mixed with other brews or conditioned in various ways. To accentuate the hop aroma some hops or hop essential oil may be added at this stage (dry hopping).

Bittering of beer can also be achieved by using pre-isomerized hop extracts. In these preparations the alpha acids have already been isomerized into iso-alpha acids so that wort boiling of the alpha acids is not needed. These isomerized extracts can therefore be added at a later stage in the beer production process, preferably as late as possible in order to increase their utilization yield. Notwithstanding the obvious economic advantages of using pre-isomerized hop extracts, they have not achieved

the expected success for a number of reasons. Brewers have a reputation for their aversion to change. Pre-isomerized extracts do influence beer taste and however small the change may be, this is deemed to be unacceptable. Furthermore, the quality of some commercial extracts is not what it should be. The presence of beta acids and of beta acids oxidation products must be strictly avoided, as these compounds cause an off-taste and gushing problems. Pre-isomerized extracts must therefore be analytically controlled. In the case of the beta acids problem, only modern Liquid Chromatography (LC) is capable of doing this. Pre-isomerized extracts, formulated as aqueous potassium salt solutions, may show demixing problems, giving rise to dosage difficulties. Adding and mixing the isomerized extraxts with beer is also much more difficult than might be expected.

Pre-isomerized extracts are ideally suited for correcting bitter level faults in finished beers; this application is on the increase. Furthermore, the quality of pre-isomerized formulations may be expected to improve continuously as a better understanding of the problems cited above is reached.

1.4.4. ECONOMICS AND UTILIZATION YIELD OF HOPS.

As mentioned in the preceding section, there are several ways to use hops in the brewery. The main aim is to transform hop alpha acids into beer iso-alpha acids. This transformation is characterized by a utilization yield which is usually only 20-35% depending on the beer type and the particular brewing procedure. The alpha acids losses occur during wort boiling (the isomerization yield is only 40-65%), filtering, fermentation (in the fermentation foam head) and lagering. Higher figures have been reported (25)

An improvement of this low utilization yield is possible by using milled or ground hops. Hop powder disperses more readily in the boiling wort and this accelerates the isomerization reaction (26). The improved utilization yield can thus reach 40% (27). Handling hop powder is however not so easy and therefore pelletized forms of hop powder were introduced. Hop pellets are supposed to break up readily in the boiling wort and they can be handled easily (29). Moreover, hop pellets can be stored more conveniently and in a smaller volume than hop powder or hop as such. As they are packed in aluminium foil or in polyvinylchloride (PVC) bags, this technique is claimed to improve storage characteristics markedly (30). If kept below 5°C, packaged hop pellets or hop powder do not lose alpha acids (23). The use of hop pellets is increasing sharply and had reached 1/3 of the total hop use by the end of 1980.

A special form of hop powder is made up of lupulin glands (28). By winnowing

techniques lupulin can be separated from powdered hop (31). Lupulin glands separated in this way are unavoidably crushed or slightly damaged. In this state they are very sensitive to oxidation and must be used immediately.

Hops can also be extracted with a range of solvents and a number of specialized industries are active in this field. Bitter acids are very soluble in all solvents except water and indeed a very large range of solvents has been used to produce commercial hop extracts. Methylene chloride and acetone are being discontinued for obvious reasons of toxicity and environmental concern, but methanol and hexane, which are still used today, are under criticism for the same reasons.

Chemists will readily accept that the low residual concentration of any volatile solvent in an extract will not leave even a trace in beer because about 10-15% of the wort is distilled away in the wort boiling process. Obviously, this will remove all possible solvent by the steam distillation effect.

The hop essential oil component myrcene, an obnoxious chemical and in a sense a 'solvent', is present in correctly-made hop extracts in concentrations which can reach several percent. The same steam distillation effect removes all traces of myrcene from wort which is boiled for 2 hours. If this were not the case, the result would be disastrous. Sub-ppb levels of myrcene may impart a hoppy flavour to beer, but ppm levels of myrcene (amount added by the hop or hop extract) would be very disagreeable. If a hoppy flavour, maybe involving extremely small traces of myrcene, is desired, very late hopping or cold hopping have to be used.

This example is discussed at some length in order to stress the harmlessness of some residual solvent in hop extracts. Still, the public at large has not the informed insight of the chemist, and popular feeling against 'chemical meddling with the quality of life' has become so strong that it is better to avoid even traces of solvents in hop extracts. The only acceptable solvents to extract hop today seem therefore to be water, ethanol and supercritical carbon dioxide. Indeed, these chemicals are found in high concentration in beer anyway and therefore, extracts which add eventually some ethanol or carbon dioxide to beer, are considered to be harmless.

Water will not dissolve the hop bitter acids but it can extract several other hop extractables (tannins, some hop bitter acids oxidation products, proteins, carbohydrates and salts). Water is therefore sometimes used to extract apolar-solvent-extracted-hops. The result is a so-called kettle additive. Hop extracts can be diluted or conditioned with such 'kettle additive' to achieve a given alpha acids content.

Ethanol and supercritical carbon dioxide easily dissolve both alpha and beta

acids. With the large difference in polarity of these solvents (apolar supercritical carbon dioxide versus polar ethanol) the composition of both extract forms will be very different. Ethanol will extract up to 50% of the hop weight, while supercritical carbon dioxide extracts only about 15-25%. The alpha acids content of supercritical carbon dioxide extracts will therefore be much higher than that of extracts obtained with polar solvents like ethanol.

Moreover, removal of ethanol from the extracts needs heating at relatively high temperature leading to partial heat isomerization of the alpha acids. This may be considered to be beneficial as the alpha acids have in any case to be isomerized, but heat may also cause other reactions of the sensitive hop bitter acids and high temperatures must therefore be avoided. Still, the presence of iso-alpha acids in ethanol extracts constitutes an additional challenge in the evaluation of such extracts, which is only possible by LC (32). This point is discussed more fully in Chapters 15-17. It follows that there is a wide range of hop extracts on the market, varying widely in their alpha acids content (31).

Supercritical carbon dioxide extracts up to 99% of the alpha acids and the extracts have usually a yellow-golden colour (33,34). The absence of tannins, hard resins and salts may be considered to be an advantage since all non-bitter contributions to beer should be avoided. Alternatively, it may be estimated that such extracts deviate too much from the original hop composition to be true to nature. A defendable view contends that much more than the alpha acids, if not all the hops extractables, will impart a more full-bodied flavour, closer to the original flavour obtained with whole hops. Counter-arguments will invoke 'harsh bitter' from non-alpha acids contributions and will state : "The purer the alpha acids form used for brewing, the purer the bitter taste obtained".

Any extraction procedure will eliminate a large amount of material which is otherwise boiled with the wort. The alpha acids content of extracts is higher than that of hops. Thus, extraction eradicates many of the differences between hop varieties. This means that brewers, who would not use certain hop varieties as such (estimating that they are of insufficient quality), will use extracts produced from these hop varieties (estimating that the quality of extracts is mainly determined by their alpha acids content and that the origin of the extracted hop is not so important). The practice of years in many breweries shows that this policy is sound.

Milling and pelletizing hops destroy the lupulin glands and therefore, promote oxidation. Storing these hop formulations without change must be difficult, in spite of contrary assertions in the literature. Hop extracts, on the contrary, can be stored easily

and do not need refrigeration as long as the tins have not been opened. Moreover, the volume of extracts is much smaller than that of hops. These are important points for many breweries. Extracts also give an increased utilization yield of the alpha acids (up to 40%) and therefore, it is not surprising that most beers today are bittered with hop extracts. There is a vast literature on hop extracts. For a fairly recent review we refer to the paper by Strauss on the past, present and future of hop extracts (35). Carbon dioxide extraction of hops continues to attract much attention (36-40).

The utilization yield with pre-isomerized extracts can be as high as 80-90%, but these data have not been substantiated by practice over long periods. Isomerization of each alpha acid gives rise to two iso-alpha acids, a cis form and a trans form. There seems to be a small difference as regards these two forms of iso-alpha acids in the intensity and the nature of the bitter taste. The ratio of these cis and trans compounds in isomerization mixtures depends on the production method. This is an extra complication which needs careful consideration. Pre-isomerization of alpha acids with alkali or in the presence of some bivalent metal-ions leads to the formation of undesirable compounds, like the allo-iso-alpha acids, acetylhumulinic acids or humulinic acids.

On the other hand, the hop bitter acids constitute such a small fraction of the beer cost that a gain in utilization yield is not relevant with respect to a change in beer quality. In our opinion there is little chance that isomerized extracts will ever conquer the market. However, isomerized extracts can help though to correct undesirable situations for instance to increase the bitter level of beers which, for some reason are not bitter enough.

It might be of interest to experiment with isomerized extracts in the wort boiling stage. The dangers of some isomerized extracts (beta acids) would be automatically avoided. The utilization yield would be low, but of all the various hop preparations, isomerized extracts contain least of the possibly undesirable hop fractions.

Efforts are also being made to find new outlets for isomerized extracts. Apparently many drinks and dairy products are improved by the addition of a faint bitter taste (41). This seems worth looking into. At one time we added pure trans isohumulone to Coca Cola with quite interesting results.

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CHAPTER 2
THE ALPHA ACIDS

The alpha acids are the most important constituents of hops. Not surprisingly, they are also the best known hop bitter acids.

2.1. STRUCTURE OF THE ALPHA ACIDS.

The fraction of the hop constituents which is precipitated by adding lead(II) acetate is commonly referred to as the hop alpha acids (1). The exact structure of the yellow-coloured lead salts is not known with certainty. However, the tertiary alcohol function of the alpha acids (3 , Fig. 2) must be involved in the complex binding, as the hop beta acids do not form such lead salts. It has been established that a 1:1 complex is formed (2).

Lead salt formation of the alpha acids.

A hop extract, largely free of solvent, is dissolved in ten times its volume of methanol. After standing overnight at 0°C the hop waxes are precipitated and can be removed by centrifugation. A methanol solution of lead(II) acetate (10% excess with respect to the total amount of hop alpha acids as determined by analysis) is prepared. The two solutions are combined whereby the lead salts of the alpha acids are precipitated. After centrifugation, the precipitate is washed repeatedly with benzene and methanol. The lead salts may be green coloured. Interfering chlorophyll colour may be removed first by passing a benzene solution of the hop extract through a dry silica gel column. Lead salt made from such purified solutions have a canary yellow colour.

2.1.1. HUMULONE, THE MAIN ALPHA ACID.

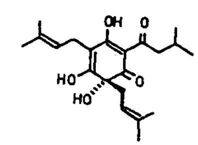

Fig.1. Humulone (3).